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Astaxanthin Production by Freshwater Microalgae *Chlorella sorokiniana* and Marine Microalgae *Tetraselmis* sp.

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Abstract: There are numerous commercial applications of microalgae nowadays owing to their vast biotechnological and economical potential. Indisputably, astaxanthin is one of the high value product synthesized by microalgae and is achieving commercial success. Astaxanthin is a keto-carotenoid pigment found in many aquatic animals including microalgae. Astaxanthin cannot be synthesized by animals and provided in the diet is compulsory. In this study, the production of astaxanthin by the freshwater microalgae *Chlorella sorokiniana* and marine microalgae *Tetraselmis* sp. were studied. The relationship between growth and astaxanthin production by marine and freshwater microalgae cultivated under various carbon sources and concentrations, environmental conditions and nitrate concentrations was investigated in this study. Inorganic carbon source and low nitrate concentration favored the growth and production of astaxanthin by the marine microalgae *Tetraselmis* sp. and the freshwater microalgae *Chlorella sorokiniana*. Outdoor cultivation enhanced the growth of microalgae, while indoor cultivation promoted the formation of astaxanthin. The results indicated that supplementation of light, inorganic carbon and nitrate could be effectively manipulated to enhance the production of astaxanthin by both microalgae studied.

Key words: Astaxanthin, *Chlorella sorokiniana*, *Tetraselmis* sp., marine microalgae, freshwater microalgae

INTRODUCTION

Astaxanthin is an important keto-carotenoid pigment that has been widely applied in the cosmetics, food and feed industries. Astaxanthin is ubiquitous in nature and has been used to impart a pinkish red color to the flesh of aquatic animals. Astaxanthin cannot be synthesized by animals and must be provided in their diet (Kim *et al*., 2006). Some natural sources of astaxanthin include microalgae, salmon, plankton, arctic shrimp and *Phaffia rhodozyma* yeast. Among these sources, microalgae appear to be the richest commercial source for natural astaxanthin (Goswami *et al*., 2010).

Astaxanthin is gaining importance in research owing to its superiority over other carotenoids as an antioxidant, anticancer agent and pigment producer. Astaxanthin is a powerful bioactive antioxidant and its efficacy has been demonstrated in animal and human models of macular degeneration (Snodderly, 1995). The biotechnological production of astaxanthin using microalgae is advantageous over chemical synthesis or extraction from crustaceans (Goswami *et al*., 2010). The increasing concerns for consumer safety and regulatory issues regarding the introduction of synthetic chemicals into the human food chain have further enhanced interest in the synthesis of natural astaxanthin (Capelli and Cysewski, 2007). This study was conducted to investigate the formation of astaxanthin in the microalgae *Chlorella sorokiniana* and *Tetraselmis* sp. cultivated under carbon, glucose and light conditions that were optimized to facilitate high-yield production of astaxanthin.

MATERIALS AND METHODS

Algae and culture conditions: The fresh water microalgae *Chlorella sorokiniana* and the marine microalgae *Tetraselmis* sp. were obtained from Algaetech Ltd., Malaysia. The freshwater microalgae *Chlorella sorokiniana* and the marine microalgae *Tetraselmis* sp. were cultivated in proteose medium and F/2 medium, respectively. The media were prepared according to the formulations available via the UTEX, The Culture Collection of Algae at The University of Texas at Austin website (http://www.sbs.utexas.edu/utex/). To determine the effects of different carbon sources on the growth and production of astaxanthin by microalgae, the organisms were cultivated in the presence of organic carbon (glucose) and inorganic carbon (CO_2). To study the effects of different light sources, the microalgae were

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cultivated under two different conditions, indoor cultivation in the lab under fluorescent light with continuous illumination with intensity of 22.25 μmol m^{-2} sec^{-1} and outdoor cultivation in an enclosed area outside the lab where the microalgae could obtain adequate sunlight. To investigate the effects of nitrate on growth and astaxanthin production, the microalgae were cultivated in medium containing different nitrate concentrations (0.14, 0.27, 0.40 and 0.55 g L^{-1}). The medium pH was adjusted to 6.5 and 10% inoculums were added to each flask.

Determination of astaxanthin: To measure the astaxanthin, 5 mL of microalgae cultured under different conditions were taken and centrifuged for 5 min at 2000 xg. The pellet was then saponified by the addition of 5% KOH in 30% (v/v) methanol rat 70°C for 5 min to destroy the chlorophyll. Following saponification, the supernatant was discarded and two drops of acetic acid were added to reduce the pH. The pellet was then extracted twice in 3 mL DMSO/acetone at 70°C for 5 min to recover the astaxanthin. The absorbance of the combined extracts was measured at 490 nm. Readings were taken on days 14, 20 and 28.

RESULTS

Effect of carbon dioxide on growth and astaxanthin production: The effects of inorganic carbon (CO_2) on the growth and astaxanthin production of both microalgae were also investigated. The effects of aeration by air sparging the culture were investigated in the early stages of the experiment. During cultivation, two types of aeration systems were applied, vigorous bubbling (100 bubbles/min) and slow bubbling (60 bubbles/min). Vigorous bubbling supplied more CO_2 to the cultures than slow bubbling. A culture that was not aerated served as the control. Vigorous bubbling was found to induce higher astaxanthin production by *Chlorella sorokiniana* than slow bubbling. However, the amount of astaxanthin produced by *Tetraselmis* sp. did not differ between cultures subjected to vigorous and slow bubbling as shown in Fig. 1.

Effect of environmental conditions on growth and astaxanthin production: Two different environmental conditions (outdoor and indoor) were applied to study the effects of different light conditions on the growth and production of astaxanthin by microalgae. As shown in Fig. 2, outdoor cultivation promoted the growth of both microalgae compared to indoor cultivation. *Chlorella sorokiniana* reached peak of exponential phase

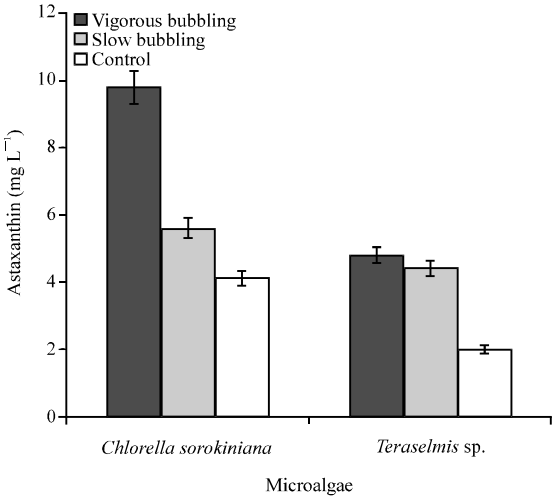

Fig. 1: The effect of air bubbling rate on the production of astaxanthin by microalgae *Chlorella sorokiniana* and *Tetraselmis* sp.

at day 15 whereas *Tetraselmis* sp. took 25 days to reach the peak exponential phase in both cultivation condition. This could be due to the greater potential of *Chlorella sorokiniana* to utilize the nutrients in the medium to sustain faster growth as compared to *Tetraselmis* sp.

Figure 3 shows the effects of outdoor and indoor culture on astaxanthin accumulation in the cells. Astaxanthin was shown to be produced higher in the indoor environment compared to the outdoor. It can be confirmed that although outdoor cultivation of microalgae promoted the growth of both microalgae *Chlorella sorokiniana* and *Tetraselmis* sp. , the condition however did not promote the production of astaxanthin.

Effect of nitrate concentration on growth and astaxanthin production: The effects of nitrate on growth and astaxanthin biosynthesis were studied by adding different concentrations of $NaNO_3$ nitrate (0.14, 0.27, 0.40 and 0.55 g L^{-1}) to the culture media containing the microalgae. As shown in Fig. 4 and 5, the growth and astaxanthin production of both microalgae were enhanced by 0.14 g L^{-1} nitrate concentration.

DISCUSSION

The supplementation of inorganic carbon to the culture enhanced the production of astaxanthin by microalgae as shown in this study (Fig. 1). Dong and Zhao (2004) reported that microalgae haveMarch 6, 2013 a very high CO_2 fixation ability and are able to tolerate CO_2 levels as high as 12%. Accordingly,

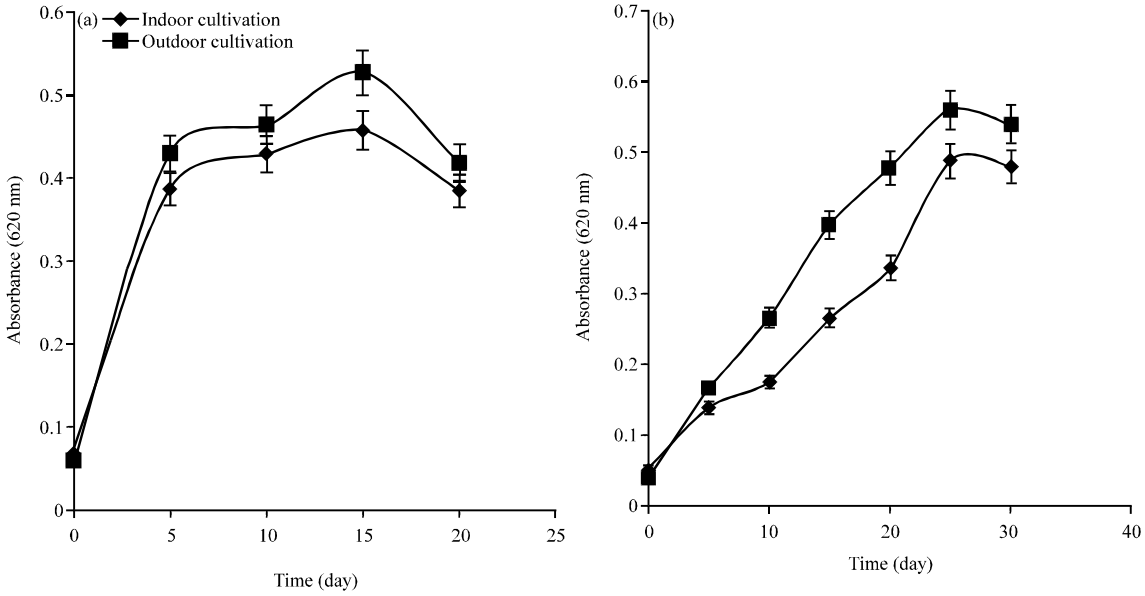

Fig. 2(a-b): The growth curve of (a) *Chlorella sorokiniana* and (b) *Tetraselmis* sp. in different environmental conditions (indoor and outdoor)

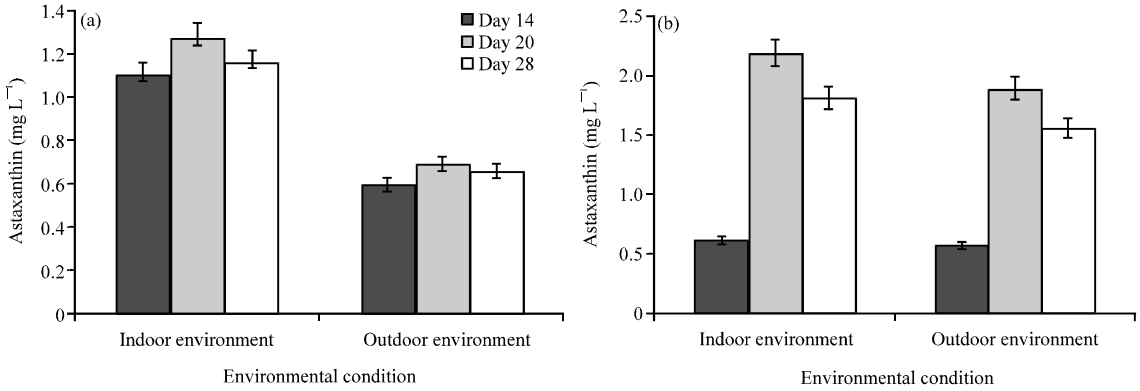

Fig. 3(a-b): Effect of different environmental conditions (indoor and outdoor) on the production of astaxanthin by (a) *Chlorella sorokiniana* and (b) *Tetraselmis* sp.

microalgae can convert CO_2 in the air into valuable biomass such as astaxanthin, as illustrated by the enhanced production of astaxanthin observed in the aerated cultures in the present study. Cultivation under the optimized conditions also resulted in improved growth and production of astaxanthin by *Chlorella sorokiniana* and *Tetraselmis* sp.

The production of astaxanthin by both microalgae under different environmental conditions was measured on days 14, 20 and 28. These days were selected owing to the fact that astaxanthin accumulation is highest around the exponential growth phase of microalgae. The results revealed that indoor cultivation promoted the production of astaxanthin by both microalgae. These findings were contrary to the greater growth of microalgae observed during outdoor cultivation. According to Imamoglu *et al.* (2009), light is essential for astaxanthin formation and growth of the alga. However, continuous illumination rather than light/dark illumination cycles has been shown to be more favorable for astaxanthin production by microalgae, indicating that light quantity is more important than light intensity for production of this compound (Kobayashi *et al.*, 1992). The use of continuous illumination instead of light/dark cycles might represent an additional source of stress that could accelerate the process of astaxanthin accumulation (Fabregas *et al.*, 2001).

As shown in Fig. 4 and 5, the growth and astaxanthin production of both microalgae were enhanced by 0.14 g L^{-1} nitrate. According to Ip *et al.* (2004),

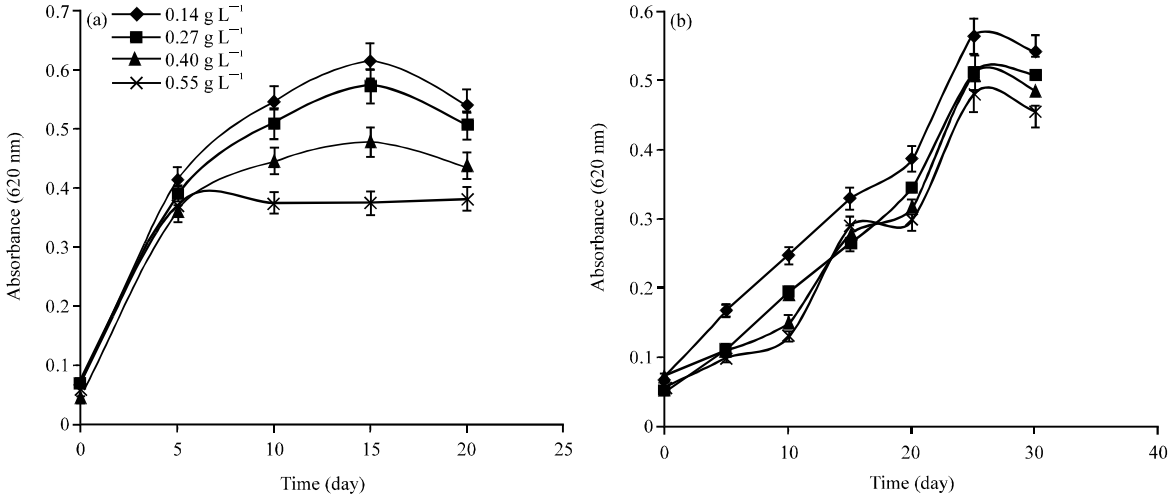

Fig. 4(a-b): The growth curve of (a) *Chlorella sorokiniana* and (b) *Tetraselmis* sp. in different nitrate concentrations

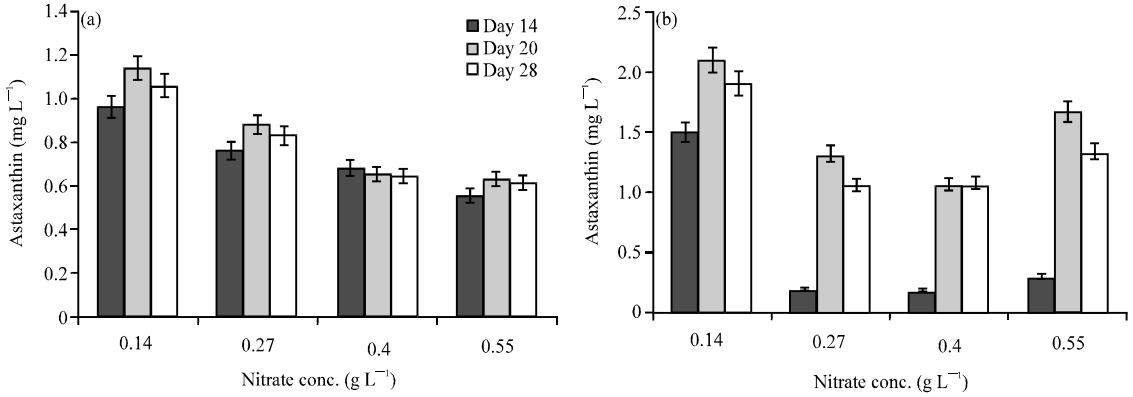

Fig. 5(a-b): Effect of nitrate concentration on production of astaxanthin by (a) *Chlorella sorokiniana* and (b) *Tetraselmis* sp.

astaxanthin is a secondary carotenoid. It is well known that secondary carotenoids such as astaxanthin and canthaxanthin can only be synthesized in algal cells when chlorophylls and primary carotenoids are not sufficient to protect the algae against environmental stresses such as high light intensity and nitrogen starvation (Rice *et al.*, 1994). In the present study, 0.14 g L^{-1} of nitrate likely resembled nitrogen starvation and led to the reduction of primary metabolism, which in turn triggered secondary metabolism to support the biosynthesis of astaxanthin.

CONCLUSION

The present study has shown the potential of *Chlorella sorokiniana* and *Tetraselmis* sp. to produce astaxanthin. Aeration was identified to correspondingly influence the astaxanthin production in *Chlorella sorokiniana*. However, *Tetraselmis* sp. showed no significant difference in astaxanthin production in either high or low bubble aeration. It was also discovered from this study that indoor environment enhanced astaxanthin production in *Chlorella sorokiniana* and *Tetraselmis* sp. Both cultures have also shown that nitrate starvation yielded higher production of astaxanthin.

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